Vaccinations in autoimmune myasthenia gravis

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ENGLISH SUMMARY

Myasthenia gravis (MG) is an acquired autoimmune disease of the neuromuscular junction. The initial trigger for making the pathogenic anti-AChR antibodies which cause myasthenia gravis still has to be elucidated. As described in **Chapter 2**, anti-AChR antibodies can be present long before clinical onset of the disease. We described a unique case of a young female with MG in whom serum samples were available over a period of at least 2 years before the onset of clinical symptoms. This patient showed a gradual increase of anti-AChR antibodies in a period of more than two years before becoming symptomatic of myasthenia. Our data suggest that pregnancy triggered the clinical manifestation of a smouldering autoimmune antibody response, but was not the primary trigger that started the production of anti-AChR antibodies in itself.

In all human clinical trials, good, validated, clinical outcome measures are of great importance. One of these outcome measures in myasthenia gravis is the 15-item Myasthenia Gravis Quality of Life (MG-QoL15). The MG-QoL15 scale has been developed to assess the health-related quality of life of patients with MG. The aim of this study was to translate the original English version into Dutch and to test the test-retest reliability and construct validity **(Chapter 3)**. Fifty patients with MG were included. Test-retest reliability and internal consistency were assessed using the intraclass correlation coefficient (ICC) and the Cronbach α. Construct validity was assessed by testing 5 predefined hypotheses, which were defined based on previous literature and the content of the outcome measure. A good test-retest reliability was confirmed with an ICC of 0.866. The Cronbach α was 0.93. The predefined hypotheses were confirmed in 80% of cases, which points to good construct validity. Since the questionnaire is validated in Dutch, it can be used for research in a Dutch-speaking population. It is also suitable for monitoring individual patients in clinical practice **(Chapter 3)**.

Patients with an autoimmune disorder are believed to be at an increased risk of infection, due to their immunosuppressive therapy or due to the immune abnormalities associated with their disease. Therefore, patients with an autoimmune disease, like myasthenia gravis (MG), are recommended to use preventive vaccinations, in particular the influenza vaccination. Since MG is a rare disease, specific studies on the effect of vaccinations in MG were not yet performed.

In **Chapter 4** we described a prospective study on the efficacy and safety of tetanus revaccination in patients with stable MG or Lambert-Eaton myasthenic syndrome. The aim of this study was to investigate the humoral immune response to and safety of a tetanus revaccination in these patients. A tetanus revaccination was administered to 66 patients. Before and 4 weeks after revaccination a blood sample and clinical outcome scores were obtained. Anti-tetanus IgG total, IgG1 and IgG4 titres were measured with an ELISA and disease-specific antibody titres (AChR, MuSK or VGCC) with a radio-immunoprecipitation assay. A historic healthy control group was used

for comparing tetanus antibody titres with that of our patients. A placebo (saline) vaccination group was used to investigate the variability of clinical outcome scores with a 4 weeks interval.

In 60 of 65 patients, we found a significant increase of the anti-tetanus antibody response. Thymectomy did not have an impact on this responsiveness. Patients with immunosuppressive medication had a significantly lower pre and post titre compared to healthy controls, but their response was still significant. The titrers of disease-specific antibodies were unchanged 4 weeks after revaccination. The clinical outcome scores showed no exacerbation of symptoms of the disease.

Therefore, we concluded that a tetanus revaccination in patients with myasthenia gravis or Lambert-Eaton myasthenic syndrome is safe and induces a significant immune response, irrespectively of their immunosuppressive medication. We observed neither immunological nor clinical relevant exacerbations associated with the tetanus revaccination.

A small number of observational studies suggest that influenza vaccination is safe(1-3) and recently a randomized controlled trial showed that influenza vaccination has no influence on AChR antibody titres.

In the Netherlands, annual vaccination against influenza is recommended for all patients with an autoimmune disease. However, in our personal experience and as described earlier, many patients express concern that vaccination may lead to an exacerbation and a substantial number decline vaccination each year based on these concerns. This is unfortunate, as seasonal vaccination against influenza is highly effective in reducing laboratory-confirmed influenza illness, hospital admissions and risk of death, especially in elderly and frail patients. This is relevant, as this age group has the highest incidence of autoimmune MG. In Chapter 5 we describe our prospective, placebo-controlled study on influenza vaccination in AChR MG. The aim of this study was to investigate the efficacy and safety of an influenza vaccination in patients with AChR MG. An influenza vaccination or placebo was administered to 47 AChR MG patients. Before and 4 weeks after administration blood samples and clinical outcome scores were obtained. Antibodies to the vaccine strains A/California/7/2009 (H1N1) pdm09, A/Hong Kong/4801/14 (H3N2) and B/Brisbane/060/08 were measured using the hemagglutination-inhibition (HI) assay and disease-specific AChR antibody titres were measured with a radio-immunoprecipitation assay. Forty-seven healthy controls (HC) were vaccinated with the same influenza vaccine to compare antibody titres. A post-vaccination, seroprotective titre (HI≥1:40) was achieved in 89.4% of MG patients vs. 93.6% in healthy controls for the H3N2 strain, 95.7% vs 97.9% for the H1N1 strain and 46.8 vs 51% for the B-strain. A seroprotective titre for all three strains of the seasonal influenza vaccine was reached in 40.4% (19/47) of the MG group and in 51% (24/47) of the HC group. Immunosuppressive medication did not significantly influence post geomean titres (GMT). The titres of disease-specific AChR antibodies were unchanged 4 weeks after vaccination. The clinical outcome scores

showed no exacerbation of MG symptoms. Concluding, the antibody response to an influenza vaccination in patients with AChR MG was not different from that in healthy subjects, even in AChR MG patients using immunosuppressive medication. Influenza vaccination does not induce an immunological or clinical exacerbation of AChR MG.

In **Chapter 6** we describe the cellular response to a tetanus revaccination, combined with broad subsets of T- and B-cells before and after vaccination. The fifty, included patients are the same as the study that investigated the humoral response to tetanus vaccination in Chapter 4. Before and 4 weeks after revaccination a blood sample was obtained. Lymphocyte subsets and B- and T-cell differentiation stages in isolated peripheral blood mononuclear cells (PBMC) were investigated by flowcytometry. PBMC were in vitro stimulated with TT (0.2 or 20 Lf/mL) and, after 3H-thymidine uptake, a stimulation index (SI) ≥3.0 was considered as evidence of antigen-induced proliferation.

Patients showed a significant tetanus induced proliferative response. Lower pre and post vaccination SI was found in patient with immunosuppressive medication (IM+) compared to those without IM (IM-). Despite this, both groups reached a significant post vaccination response. TT revaccination did not affect cell counts of lymphocyte subpopulations and B- and T-cell differentiation stages. A preceding thymectomy showed no effect on lymphocyte compartments. However, IM, in particular azathioprine, was associated with strongly decreased NK cell and B-cell counts, but did not affect levels of anti-TT antibodies before or after revaccination.

Overall, we concluded that, TT revaccination resulted in an increase of the in vitro tetanus-specific proliferative response and did not affect the composition of lymphocyte compartments. Whereas thymectomy had no significant influence, significant effect of immunosuppressive medication, azathioprine in particular, i.e. a decrease of numbers of B-cell subsets and NK cells, was found. However, this had no impact on the IgG anti-tetanus response upon revaccination. In conclusion, revaccination is effective in adult AChR MG patients with stable disease irrespective of their thymectomy status and actual immunosuppressive medication.